relieve the inhibition. The goal of this study is to elucidate the structural rearrangements during relief of inhibition. Previous EPR and NMR studies found that the cytoplasmic domain of PLB exists in a dynamic equilibrium between T and R states. We have used time-resolved fluorescence resonance energy transfer (TR-FRET) to probe the complex directly by measuring the distances between SERCA (C674) and a series of positions on PLB in co-reconstituted vesicles. We found that the distances between SERCA and the cytoplasmic domain of PLB are best fitted with two Gaussian distance distributions, suggesting that the cytoplasmic domain of bound PLB exists in two states. Results will be reported on the effects of perturbations in this system which are known to relieve inhibition, including PLB phosphorylation, PLB mutation, and calcium binding to SERCA. We conclude that the function of the SERCA-PLB complex depends primarily on the structural equilibrium between two principal structural states of the complex.

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Probing Conformational Changes of the Na^+/K^+ ATPase upon Ouabain Binding by using a Spectroscopic Approach

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The Na^+/K^+ -ATPase is an integral membrane protein responsible for Na^+ and K^+ homeostasis, thereby fundamental for cellular life. It is formed mainly by two subunits: α and β. Ouabain, a cardiotonic derivative, binds to the ion permeation pathway of the Na^+/K^+ -ATPase within the α subunit. To gain further insights into the ouabain binding mechanism, we used a spectroscopic approach (LRET) to measure distances between a genetically encoded lanthanide binding tag (LBT; that binds Tb^{3+} with high affinity) and a cysteine-reactive fluorescent compound (Bodipy). We created energy transfer pairs by encoding LBTs in three of the external loops of the α subunit, connecting transmembrane helices (TM) 1-2, 3-4 and 9-10, and cysteines substituted in different positions of the β subunit. These constructs were expressed in *Xenopus* oocytes. Distances were estimated using the luminescence decays of donor only and sensitized emission, upon excitation with a 9-ns pulse at 266 nm. In each oocyte expressing a LBT-(Tb^{3+})-Bodipy pair, distance determinations were made in the absence, and in the presence of ouabain. The results suggest that upon binding of ouabain, all external loops move as if the extracellular end of the permeation pathway of the pump opens up in the presence of ouabain. Further, no significant movements of the β subunit were detected in the LRET experiments. With these determinations, molecular modeling of the LBT-(Tb^{3+})-Bodipy pair constructs were performed to build models corresponding to pump's conformations in the absence and presence of ouabain. These models suggest partial opening of the TM helices on the extracellular side. These changes will allow access of ouabain to its binding site, deep inside the permeation pathway. Supported by U54GM087519, GM030376 and NIH/NINDS Intramural Program.

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Listeria Monocytogenes Lmo0818 - Exploring a Putative Ca^{2+}-ATPase, to Understand Calcium Ion Specificity

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This project focus on calcium homeostasis in the opportunistic pathogenic bacteria Listeria monocytogenes. Genomic analysis of L. monocytogenes identifies two P-type ATPases with putative selectivity for Calcium transport (Lmo0841 and Lmo0818). Lmo0841 was recently confirmed as a Ca^{2+} -ATPase, able to transport a single Calcium ion per hydrolyzed ATP the L. monocytogenes Ca^{2+} -ATPase was consequently named LMCA1 [1] Sequence analysis and homology modeling strongly suggest that Lmo818 is a Ca^{2+}ATPase with high similarity to the plasma membrane ATPases (PMCA), a type IIb P-type ATPase from higher eukaryotes. There is no X-ray structures of any PMCA's determined, they are known to be less stabile in comparison with the sarco(endo)plasmic reticulum Ca^{2+} -ATPases (SERCA) and also often require additional soluble factors to function, like the necessity of Calmodulin to the PMCA [2]. We have determined the structure to 2.7 Å resolution initial structural comparison indicate a similar fold to SERCA, however with significantly reduced loop regions. A comparative bioinformatics study will be presented based on Lmo0818 that reveal how the pumps are could act as receptors in prokaryotic membrane systems as compared to ion recognition and receptor function found in the Na+,K+ -ATPase [3].

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An Arginine-Agmatine Antiporter Optimized for Extreme Acid Resistance in Enteric Bacteria

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Enteric bacteria such as E. coli and Salmonella species can maintain cytoplasmic pH in the extremely acidic gastric environments by consuming intracellular protons for arginine decarboxylation. This reaction is kept energetically favorable by an APC (amino-acid-polyamine-organocation) family antiporter AdiC, which imports arginine while pumping out the decarboxylated product agmatine in a 1:1 exchange stoichiometry. To characterize AdiC's transport behavior in extreme acid conditions, we developed an oriented reconstituted liposome system able to hold a 3-unit transmembrane pH gradient. Our work reveals two unique functional properties by which AdiC maximizes its effectiveness in conferring acid resistance. First, AdiC possesses a pH-sensing mechanism that permits maximal transport activity only under intra- and extracellular pH conditions of bacteria experiencing strong acid stress (i.e., outside pH 2-3; inside pH 4.5-5.5). Second, we demonstrate that AdiC mainly catalyzes electropositive exchange between extracellular arginine (50% Arg^+, 50% $Arg2^+$, pH2.2) and cytoplasmic agmatine ($Agm2^+$, pH5.5) - the exchange is strongly inhibited by the imposition of negative membrane potentials. Thus, at gastric pH, AdiC selectively imports the less common Arg^+ against the useless α-carboxyl protonated $Arg2^+$, whose deprotonation upon entry into the cytosol would cancel out the proton-removing effect of arginine decarboxylation. We are currently investigating the underlying mechanisms for this second feature of AdiC. Preliminary results from testing various arginine analogues suggest that AdiC differentiates Arg^+ and $Arg2^+$ based on their difference in valence number rather than the protonation status in the α-carboxyl.

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Structure and Conformation of Rhizoferrin and its Fe^{3+} Complexes

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Rhizoferrin is a simple but efficient siderophore and is potentially important for medical applications. The aim of this investigation is to determine the molecular structure of free rhizoferrin as well as its Fe^{3+} complexes, and to understand the mechanisms of cation (Fe^{3+})-binding specificity of rhizoferrin, using first-principle methods. The optimized molecular structures, and the calculated Nuclear Magnetic Resonance properties, such as chemical shifts for ^{13}C and ^{1}H and indirect dipole dipolecouplings for ^{1}H, will be presented and compared with the experimental data. The nuclear quadrupole interaction parameters will be discussed as an attempt to understand the charge distribution and electric field gradient. Possible influence of deprotonation of citrate carboxylate groups on Fe^{3+}-rhizoferrin complex formation will be discussed. This is, to the best of our knowledge, the first theoretical study of rhizoferrin and its metal complexes, and it provides a critical insight and guidance to the structural basis of metal chelation by rhizoferrin.

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Supported Membrane System to Evaluate the Ion-Pump Activity of Reconstituted Proteins under an Optical Microscope

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Supported membrane is the experimental platform in which a lipid bilayer is tethered on a solid surface. Owing to the mechanical stability, it allows us to investigate physicochemical properties of membranes and also membrane proteins reconstituted in it. In this study, we developed a novel supported membrane system that enables to measure the ion-pump activity of membrane proteins under an optical microscope. As a model ion pump, we reconstituted FoF1-ATP synthase (FoF1) in liposomes. To prepare the supported membrane on a cover slip, we deposited the liposome on the cover slip and dehydrated them. Sequentially we rehydrated it with the buffer. This is relatively simple way to generate an artificial bilayer under optical microscope. In the rehydration buffer, we contained a pH indicator fluorescent dye (pHrodo) and Caged-ATP. By introducing the evanescent illumination of 405 nm light for uncaging of ATP from the bottom of the glass surface, ATP was supplied only in the illumination area. The local pH of the membrane-surface space was monitored with the fluorescent signal of pHrodo excited with the evanescent illumination of 532 nm. Only after the illumination for uncaging, the intensity of pHrodo